

Induction of recombination by 4-OH Tamoxifen intraperitoneal injection in adult zebrafish

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An abbreviated version of this protocol was published in eLIFE

Zebrafish fin regeneration involves generic and regeneration-specific osteoblast injury responses

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Detailed protocol

This is a step-by-step protocol for the induction of recombination by 4-OH Tamoxifen intraperitoneal injection in adult zebrafish.

1) Preparation of 4-OH Tamoxifen stock

It's best to use freshly dissolved 4-OH Tamoxifen. Aliquots of dissolved solutions kept at -20°C are stable for up to 3 months. It is not recommended to use older dissolved aliquots.

- Use (Z)-4-Hydroxytamoxifen (4-OHT) which contains > 98 % Z-isomer
- The final concentration of the stock should be 10 mM, the solvent is 100% EtOH.
- Prepare 20 µl aliquots. Store aliquots at -20°C.
 - *It is best to use PCR tubes. In larger tubes, the injection needle might not reach the bottom.*
- Use aliquots of 4-OH Tam dissolved in EtOH only once and DO NOT re-freeze an aliquot.

2) 4-OH Tamoxifen intraperitoneal injection in adult zebrafish

- Injection volume: 10 µl
- Injection concentration: 3.4 mM

Injection regime

- How many injections are needed for efficient recombination and in which time-frame they should be applied will depend on the CreERT2 line.
- Four injections appear to be sufficient for most lines.
- Fish can tolerate consecutive injections every 24 hours for few days, but a day of rest every 2-3 injections is recommended.

Preparation of the injection mix

- It is important to inject the diluted 4-OHT within 10 min, otherwise the solution turns clear which is not good. **Thus, prepare everything for injection before you dilute the 4-OHT stock.**
- Make sure there are no precipitates when using an aliquot.
- Prepare the 3.4 mM injection mix by diluting with PBS.
- The solution will turn milky, which is normal. *If clear solution appears and precipitate collects at the bottom of the tube, it probably means the 4-OHT has precipitated. In this case, vortex!*

I.P. injection

- Use a 10 µl or 100 µl syringe with 33 GA needles.
Using 10 or 100 µl syringe depends on personal preference.
- Use a soft sponge with an incision as fish holder. Soak the sponge with system water, and put it into a 60 mm Petri dish.
- Anaesthetize fish with 625 µM tricaine.
- Clamp the anaesthetized fish belly-up into the incision. The tail should point towards the hand with which one is injecting.
- Prick into the midline between the pelvic fins. After injection of 10 µl, carefully withdraw the needle.
- Transfer the fish back to its tank.

Material

- (Z)-4-Hydroxytamoxifen
- 100 % Ethanol
- PBS
- tricaine
- fish holder: sponge with incision
- 10 µl or 100 µl syringe
- injection needles (e.g. World Precision Instruments, NanoFil NF33BV 33GA, beveled needle, NF33BV-2)

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sehring, I. M. and Weidinger, G. (2023). Induction of recombination by 4-OH Tamoxifen intraperitoneal injection in adult zebrafish. Bio-protocol Preprint. [bio-protocol.org/2222](https://doi.org/10.21956/bio-protocol.2222).
2. Zebrafish fin regeneration involves generic and regeneration-specific osteoblast injury responses. eLIFE. DOI: [10.7554/eLife.77614](https://doi.org/10.7554/eLife.77614)

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